The Future of Vaccines - Cancer Meets Infectious Diseases

IMMUNOLOGISTS, ONCOLOGISTS AND VACCINE DEVELOPERS DISCUSS COMMON THERAPEUTIC APPROACHES FOR FIGHTING CANCER AND INFECTIOUS DISEASES

April, 10-13, 2003

Semmering, Austria

ORGANIZED BY THE ASSOCIATION "VIENNA VACCINES"

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April 10-13, 2003, Semmering, Austria

Intercell has taken the initiative to found the association **"Vienna Vaccines"** to organize the multidisciplinary scientific workshop "The Future of Vaccines – Cancer Meets Infectious Diseases". **Baxter, igeneon, BioTec Area Krems, Eco Plus, SWX Swiss Exchange, UBS Warburg** and the initiative **www.innovatives-oesterreich.at** support the association.

Significance and Background

The organizer's goal is to initiate the exchange of ideas and concepts between two most important scientific fields in terms of biotechnology and medicine: cancer and infectious diseases. The significance of the workshop becomes even clearer by the fact that amazingly there are very rare occasions where scientists of the fields of cancer and infectious diseases meet in the area of immunology.

Parallels between cancer and infectious diseases

Infectious diseases such as hepatitis C, HIV, tuberculosis and malaria have become major killers worldwide, e.g. tuberculosis kills 3 million people per year. For numerous highly dangerous diseases no satisfactory treatment exists at present and antibiotics have increasingly become ineffective against a large number of germs. Against this growing threat of humanity new and effective vaccines are needed.

In parallel also cancer is a main cause of death and the results using immune therapy are promising.

Both fields – infectious diseases and cancer – are currently experiencing revolutions in that there are now tools and methods available that allow the design of completely novel vaccines. Although the reaction of the immune system to cancer and infectious diseases appears to follow closely similar routes (e.g. virus infected cells are similar to cancer cells), the infectious diseases and cancer communities rarely meet to exchange ideas and concepts.

Thank you for participating in "The Future of Vaccines".
The organizers of this meeting wish you a successful conference.

Conference Program

Thursday, April 10, 2003

12 - 4 p.m.	**Registration**
4:30 – 5:40 p.m.	**Welcome, Introductory Remarks** *Chair:* Max Birnstiel
5 – 5:30 p.m.	Alexander von Gabain: *“Vaccine Development: from Empirical Medicine to Molecularly Designed Therapy”*
6:30 p.m.	**Dinner sponsored by Chiron**
7:30 - 9 p.m.	**Keynote lectures** *Chair:* Max Birnstiel
7:40 – 8:20 p.m.	Antonio Coutinho: *"An outsider's view on the problems of vaccinology"*
8:20 - 9 p.m.	Michel Gréco: *“Vaccines in the 21st century: an exciting and difficult world”*
9 p.m.	**Get-together**

Friday, April 11, 2003

9 – 12:10 p.m.	**Session I: “Mechanism underlying immune response against infectious diseases and cancer: what can we learn for vaccine development”** *Chair:* Alexander von Gabain
9:10 - 9:30 a.m.	Staffan Normak: *“Bacterial Pathogenicity and Innate Immune Responses”*
9:40 - 10 a.m.	Shizuo Akira: *“TLR family as receptors linking innate and acquired immunity”*
10: 10 - 10: 40 a.m.	**break (30’)**
10: 40 - 11 a.m.	Elke Jäger: *“Antigen-specific Immunotherapy in Malignant Diseases”*
11:10 - 11:30 a.m.	Rolf Kiessling: *“The Her-2/neu oncogene as a target for tumor vaccination”*
11:40 - 12:00 a.m.	Rafi Ahmed: *“Immunological Memory: Remembering our pathogens”*
12:10 – 1:40 p.m.	**Lunch**
1:40 – 6 p.m.	**Session II: “Novel vaccines for infectious diseases”** *Chair:* Friedrich Dorner
1:50 – 2:10 p.m.	Peter J. Openshaw: *“Problems and Prospects for RSV Vaccine Development”*
2: 20 – 2:40 p.m.	Michael P. Manns: *“Present and Future Concepts for Prophylactic and Therapeutic Vaccines against Hepatitis C”*
2:50 – 3:10 p.m.	**break (20’)**

Session II: "Novel vaccines for infectious diseases" (continuation)

3:10 – 3:30 p.m. Noel Barrett: *"Development of a Novel Cell Culture-Derived Influenza Vaccine"*

3:40 - 4 p.m. Elaine Tuomanen: *"Respiratory Vaccines: Viruses and Pneumcoccus"*

4:10 – 4:30 p.m. **break (20')**

4:30 - 4:50 p.m. Martin Vordermeier: *„Recognition of identical promiscuous determinates by T cells from different mammalian species and the use of bioinformatics to predict bovine T cell epitopes"*

5 - 5:20 p.m. Hermann Bujard: *"A candidate for a malaria vaccine: the merozoite surface protein 1 of Plasmodium falciparum"*

5:30 - 5:50 p.m. Jeffrey W. Almond: *"Vector approaches to vaccination against infectious diseases and cancer"*

7 p.m. **Surprise event sponsored by Innogenetics and Aventis Pasteur**

Saturday, April 12, 2003

8 – 9:40 a.m. **Session III: "Cancer vaccines"**
Chair: Hans Strander

8:10 - 8:30 a.m. Georg Stingl: *"Cell-based and molecular vaccines against melanoma – studies in man and experimental animals"*

8:40 - 9:00 a.m. Kathrin Jansen: *"Status of Prophylactic Vaccines against Cervical Cancer"*

9:10 - 9:30 a.m. Hans Loibner: *"Therapeutic vaccination against epithelial cancers with surrogateantigen vaccines: preclinical and clinical results"*

9:40 – 10:10 a.m. **break (30')**

10:10– 12:10 a.m. **Session IV: "Emerging topics in vaccines"**
Chair: Ernst-Günter Afting

10:20-10:35 a.m. Erik Depla: *"E1 vaccination in chronic HCV patients: Well-Tolerated and Possible Halting of Fibrosis Progression in Patients"*

10:40-10:55 a.m. Martin Bachmann: *"Virus-like particles: Combining Innate and Adaptive Immunity for Effective Vaccination"*

11-11:15 a.m. Jean-Francois Viret: *„Recombinant live oral vaccines against bacterial enteric pathogens: the way through regulation"*

11:20 - 11:40 a.m. Brian Barber: *"Delivering T-cell Immunity with Heat Shock Proteins"*

11:50 - 12:05 a.m. Gregory Glenn: *"Transcutaneous Immunization: Skin Immunization Using a Patch"*

12:10 – 1:10 p.m. **Lunch**

1:10 - 5:30 p.m.	**Session V: „Novel vaccine strategies for infectious diseases and cancer“** *Chair:* Hamilton Smith
1:20 – 1:40 p.m.	Antonio Lanzavecchia: *"Maintenance of serological memory"*
1:50 – 2:10 p.m.	Bernard Moss: *"Poxvirus Vectors With and Without DNA Priming"*
2:20 – 2:40 p.m.	**break (20')**
2:40 - 3 p.m.	Siegfried Weiss: *"Bacteria mediated DNA transfer for gene therapy and vaccination"*
3:10-3:30 p.m.	Jeffrey Ulmer: *"Delivery systems for vaccines and adjuvants"*
3:40 - 4 p.m.	Rino Rappuoli: *"Reverse vaccinology, a genome-based approach to vaccine development"*
4:10 – 4:30 p.m.	**break (20')**
4:30 - 4:50 p.m.	Andreas Meinke: *"Identification of the "antigenome" – a novel tool for the design and development of subunit vaccines against bacterial pathogens"*
4:50 - 5:20 p.m.	Michael Buschle: *"Novel Potent Adjuvants for the Induction of Cellular and HumoraI Immune Responses"*
8 p.m.	**Gala dinner**

Sunday, April 13, 2003

Departure

Vaccine Development: from Empirical Medicine to designed Molecular Therapy

A. von Gabain

Intercell AG, Campus Vienna Biocenter 6, 1030 Vienna, Austria

Vaccination is arguably the most successful medical intervention. About 250 years ago, Lady Mary Wortley Montague described a medical procedure called “inoculation” used to protect humans against small pox that she observed in Turkey and applied to her own children in England. Edward Jenner seemingly inspired by her seminal and pioneering actions developed a relatively efficient vaccine against small pox. During the 19th century the evolution of modern microbiology led to the discovery of some of the components of the human immune system and the first knowledge-driven vaccines and sera-therapies entered the medical arena.

At the beginning of the 20th century, promising clinical results, with what may be considered as therapeutic vaccines against cancer, were described by Coley, a New York surgeon. He used extracts from Streptococcus pyrogenes and Bacillus prodigiosus to stimulate the immune system, was – however - challenged by contemporary peers claiming his studies lacked reproducibility and controls. After World War II the discovery of interferons and cytokines opened the field to stimulate the immune system for the treatment of tumors. During the last decade encouraging results with cancer vaccines have aided the vaccine field to integrate new concepts and to present novel strategies. This development has been facilitated by the emerging recombinant DNA technology and the explosive progress in our understanding of immune mechanisms leading to the eradication of pathogens and perhaps of cancer. In our opinion the development of novel prophylactic and therapeutic vaccines and - whether directed against cancer or infectious diseases - will depend on the following basic principles:

- » T-cell immunity is equally important as B-cell immunity for mounting an immune defense against microbes and cancer.
- » Dendritic cells play a central role in the presentation of antigens and the initiation of the adaptive immune response.
- » Defined and potent substances have been discovered that activate the pathways of the innate immune system leading to the adaptive immune response
- » Technologies have become available, alongside with genomics, that ease the identification of B and T-cell antigens specific for pathogens and malignant cells and, thus, permit optimization of the formulation of antigens in vaccines and
- » Vector systems have been developed that efficiently deliver antigens to the target cells.

The appearance of novel infectious diseases of global threat, like AIDS, the comeback of infectious diseases like Tuberculosis and Malaria and the limits of the existing cancer

therapies urgently need the development of novel vaccines that amalgamate latest concepts of immunology derived from studies of pathogens and cancer. Since the development also includes complicated processes and know-how rarely established in academic labs (e.g. IP, GMP manufacturing, clinical trials and proper distribution) academia, industry and non-profit organizations have to partner up in order to launch novel and potent vaccines to the benefit of human kind.

Vaccines in the 21st Century - An exciting, although difficult world

M. Gréco

Lyon, France

At the end of the eighties, the vaccine field attracted little interest. Vaccination was perceived as a sort of quasi-compulsory administrative act, mainly targeted to newborns. Its low prices drew little attention from the private sector, with the consequence that some of the main pharmaceutical companies were leaving the field, such as Glaxo and Welcome in the UK, Hoechst in Germany, etc… and the smaller players started to disappear as the activity was no longer financially sustainable for them.

Fifteen years later, the vaccine "market" has radically changed. It has strongly developed, in terms of sales, of number of products marketed or under development, or of companies involved.

In parallel, numerous questions have been raised, concerning all aspects, scientific, technical, public health or communication matters regarding vaccines and vaccination.

At the onset of the 21st Century, the Vaccine field has become really exciting, even if its development is constrained by many difficulties.

Vaccines, an exciting field

There are few economic sectors that pull together as many positive characteristics as vaccines:

- Vaccines address essential needs
- Demand is constantly growing
- Innovation perspectives are almost "boundary less"
- The field is protected by high technological entry barriers, thereby limiting competition
- The value of vaccines and vaccination is progressively better recognized.

Vaccines, a difficult field

While vaccines are an exciting field, their development and use face a number of difficulties, some linked to their technical characteristics, others being more a function of the changing "societal" environment and of its impact on Public Health policies.

Many questions remain to be addressed, whether medical, technical, financial or mediatic, but despite the many constraints, it is clear that the field holds a great future.

Bacterial Pathogenicity and Innate Immune Responses

B. Henriques Normark, B. Albiger, M. Hornef, A. Sandgren, and St. Normark

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Most infections occur at mucosal surfaces lining the urogenital, gastrointestinal and respiratory tracts. While the bladder epithelium normally should be kept sterile the urine frequently contains introduced bacteria, mainly from the intestinal tract. One issue to be discussed is therefore to what extent the bladder epithelium is capable of recognizing lipopolysacharides (LPS) of gram negative bacteria. The gastrointestinal tract normally contains an endogenous micro flora. Consequently, the epithelium must be hypo responsive to commensal bacteria but remain responsive to pathogens. The gastric epithelium is lacking Toll like receptor 4 (TLR4) and can therefore not respond to LPS. The proinflammatory activity of Helicobacter pylori therefore proceeds in a TLR4 independent manner and requires the expression from the cag-pathogenicity island of the microbe. While epithelial cells of the small intestine, exposed to the intestinal micro flora, are LPS unresponsive, murine crypt epithelial cells express TLR4. The murine small intestinal epithelial cell line m-IC(cl2) is highly responsive to LPS and expresses both CD14 and TLR4. Transcription and surface membrane staining for CD14 were up-regulated upon LPS exposure. Surprisingly, TLR4 immunostaining revealed a strictly cytoplasmic paranuclear distribution. This paranuclear compartment could be identified as the Golgi apparatus. LPS added to the supernatant was internalized by m-IC(cl2) cells and co localized with TLR4. Data will be presented to show that TLR4 in the Golgi of crypt epithelial cells is able to engage in host cell signalling upon interaction with internalized LPS. This intracellular LPS signalling allowed crypt epithelial cells to be hypo responsive to LPS coated onto latex beads but responsive to soluble LPS. The airways are frequently exposed to gram positive bacteria such as Streptococcus pneumoniae. This organism colonizes the nasopharynx in up to 50% of all children attending day care centres. However, occasionally it is capable of invading into the blood stream to cause invasive disease. Pneumococcal invasive disease depends both on bacterial attributes and host responses. We will present data to suggest a model for invasion that depends on TLR-mediated signalling.

TLR family as receptors linking innate and acquired immunity

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Toll-like receptors (TLRs) play an essential role in the detection of invading pathogen in the body. Individual TLR recognizes distinct components derived from pathogens, which is followed by production of cytokines. The TLR family harbors an extracellular leucine-rich repeat (LRR) domain as well as a cytoplasmic domain that is homologous to that of the IL-1R family. Upon stimulation, TLR recruits IRAK via adaptor MyD88, and finally induces activation of NF-κB and MAP kinases. Cytokine production in response to each TLR ligand is completely abrogated in MyD88-deficient cells, showing that MyD88 is an essential signaling molecule shared among IL-1R/Toll family. However, individual TLR exerts distinct gene expression. Evidence is accumulating, which indicates that differential utilization of several adaptor molecules provides the specificity in the TLR signaling.

Antigen-specific immunotherapy in malignant disease

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Tumor-associated antigens recognized by cellular and humoral effectors of the immune system represent potential targets for antigen-specific cancer immunotherapy. Different groups of cancer-associated antigens have been identified which induce CD8+ cytotoxic T lymphocyte responses in vitro and in vivo: 1) `Cancer Testis´ (CT) antigens, expressed in different tumors and normal testis, 2) melanocyte differentiation antigens, 3) point mutations of normal genes, 4) `self´-antigens that are overexpressed in malignant tissues, and 5) viral antigens. Clinical studies with peptides and proteins derived from these antigens have been initiated to study the efficacy of inducing specific CD8+ T cell responses in vivo. Immunological and clinical parameters for the assessment of antigen-specific immune responses have been defined, i.e. DTH- , CD8+ T cell-, autoimmmune-, and tumor regression responses. Specific DTH- and CD8+ T cell responses and tumor regressions have been observed after intradermal administration of tumor-associated peptides alone. Peptide-specific immune reactions were enhanced after using GM-CSF as a systemic adjuvant by increasing the frequency of dermal antigen-presenting Langerhans´ cells. Complete tumor regressions have been observed in the context of measurable peptide-specific CD8+ T cells. However, in single cases with disease progression after an initial tumor response, either a loss of single antigens targeted by CD8+ T cells or of the presenting MHC class I allele was detected, pointing towards immunization-induced immune escape. Ways to modulate antigen- and MHC class I expression in vivo are being evaluated to prevent immunoselection. Recently, a new CT antigen, NY-ESO-1, has been identified on the basis of spontaneous antibody responses to tumor-associated antigens (SEREX). NY-ESO-1 appears to be one of the most immunogenic antigens known today with spontaneous immune responses observed in 50 % of patients with NY-ESO-1 expressing cancers. Clinical studies have been initiated to evaluate the immune responses to vaccination with different NY-ESO-1 peptides combined with GM-CSF, and with viral constructs encoding NY-ESO-1 in relation to the clinical development.

The Her-2/neu oncogene as a target for tumor vaccination

R. Kiessling

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Her-2/neu (HER-2) is a 185 kD receptor-like glycoprotein that is overexpressed by a variety of tumors such as breast, ovarian, gastric and colorectal carcinomas. Overexpression of this oncogene is directly associated with malignant transformation of epithelial cells. The frequency of HER-2 overexpression varies among the different types of cancer, but universally represents a marker of poor prognosis. The critical role of HER-2 in epithelial oncogenesis as well as its selective overexpression on malignant tissues makes it an ideal target for immunotherapy. Antibodies and T cells reactive to HER-2 are known to naturally occur in patients with HER-2 positive tumors, confirming the immunogenicity of the molecule. Both antibodies as well as T cells reactive to HER-2 have been utilized for immunotherapy of HER-2 positive tumors. The "humanized" monoclonal antibody Herceptin has been tested in several clinical trials and found to be an effective adjuvant therapy for HER-2 positive breast and ovarian cancer patients. However, the frequency of patients responding to Herceptin is limited and a majority of patients initially responding to Herceptin develop resistance within a year of treatment. The use of vaccination strategies that generate T cell responses with or without accompanying antibody responses may serve to mitigate the problem. Various strategies for generating T cell-mediated responses against HER-2 are currently being examined in animal models or in clinical trials. The potential advantages of the various approaches to immunotherapy, their pitfalls and the mechanisms by which HER-2 positive tumors can evade immune responses will be discussed.

Immunological Memory: Remembering Our Pathogens

R. Ahmed

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Acute viral infections induce long-term humoral and cellular immunity. However, the nature of T- and B-cell memory is different. Antiviral B-cell memory is usually manifested by continuous antibody production that lasts for many years after infection or vaccination. In contrast, the effector phase of the T cell response is short-lived (a few weeks), and "memory" in the T-cell compartment results from the presence of memory T cells, which are found at higher frequencies and can respond faster and develop into effector cells (i.e., CTL or cytokine producers) more efficiently than can naïve T cells. In this talk I will discuss the following aspects of immunological memory: (1) The importance of long-lived plasma cells in maintaining humoral immunity. (2) Functional differences between naïve and memory T cells (3) Memory T cell differentiation and memory cell subsets; and (4) Protective immunity by memory CD8 T cells.

Problems and prospects for RSV vaccine development

P. JM. Openshaw

Imperial College London

Excellent vaccines are already available for many infectious diseases. However, remarkable challenges remain, particularly in developing vaccines that protect against or cure persistent viral infections. There is increasing evidence to suggest that respiratory syncytial virus (RSV) should be considered a persistent infection in some circumstances, and certainly vaccine development has been fraught with problems.

Although immune responses to virus infections are usually protective, they can also be harmful. The best-documented examples of an immune response increasing disease severity are with dengue, measles and RSV infections. Inducing a strong immune response is an essential aim of vaccination, but risks disease enhancement. Animal models strongly suggest that vaccine enhancement of RSV disease may be due to strong (and perhaps unbalanced) T cell priming as well as to the production of infection-enhancing antibody.

Enhanced disease can result from over-exuberant CD8 T cells (which cause extensive pulmonary necrosis and an acute capillary leak-like syndrome, similar to ARDS) or CD4 cells which recruit an abundant cellular infiltrate (the nature of which is dependant on the cytokine and chemokine patterns of memory T cells). In several models, formalin vaccination has been linked specifically to the induction of Th2 cells, which make IL-4 and IL-5 and induce a strong pulmonary eosinophilic response. Defining the correlates of protection and disease enhancement in man is critical to the rational development of effective, protective vaccines against RSV.

With the explosion of knowledge about the viral immunopathogenesis infections and vaccinology, it is to be hoped that vaccines will be increasingly available to prevent and treat persistent infections.

Present and future concepts for prophylactic and therapeutic vaccines against hepatitis C

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Infection with the hepatitis C virus (HCV) is one of the leading causes of end-stage liver disease and hepatocellular carcinoma. Worldwide, an estimated 170 million people are chronically infected with HCV. 13 years after the discovery of HCV, we have detailed information on prevalence, transmission, replication, natural history and pathogenesis of the virus. Most importantly, about half of infected patients can be successfully treated with pegylated interferons in combination with ribavirin. However, current treatment options are associated with significant side effects and are very costly. Not all patients may be treated due to contra-indications and for a sub-population infected with HCV genotype 1 and significant fibrosis, response rates are still below 50%. Thus, alternative treatment options are urgently needed.

Since HCV is a non-cytopathic virus in most circumstances, the immune response almost certainly plays a central role not only for the control of the infection but also for the pathogenesis of liver disease. The immune response against HCV is complex and generated by various cell types and tissues. Both, innate and adaptive immune responses contribute to the control of HCV infection. There seems to be no long-lasting protective humoral immunity against HCV. Anti-HCV antibodies decline after recovery from acute HCV infection to undetectable levels after two decades. Furthermore, it has been demonstrated that HCV may be cleared even in the absence of any humoral immunity against envelope proteins. In contrast, a multispecific, strong and maintained HCV-specific CD4+ and CD8+ T cell response has been shown to be associated with viral clearance. Non-cytolitic inhibition of viral replication by antiviral cytokines seems to be of great importance for the control of HCV. Resolution of HCV correlates with an early IFN-gamma response by CD8+ T cells while persistence of functionally impaired CD8+ T cells leads to chronic infection.

Therapeutic vaccinations are currently explored in hepatitis B and in hepatitis C to enhance not only humoral but may be even more importantly cellular immune responses. Peptide vaccines targeting CTL epitopes and DNA vaccines combined with different adjuvants (CpG-DNA, cytokines, Poly-Arginine, etc.) as well as recombinant viruses expressing HCV proteins might be promising ways to stimulate HCV-specific immune responses. We recently showed that even oral vaccination for hepatitis C using attenuated salmonella as carriers for HCV plasmids might be possible in future. However, for therapeutic vaccines several issues remain to be solved. High antigen load may contribute to anergization of virus-specific T cells. Thus, interferon alpha or new direct antiviral agents targeting viral enzymes like the HCV protease or the HCV polymerase might have to be combined with vaccines to induce a sufficient and sustained antiviral T cell response. If this problem has been answered we will have to address more questions: How often do we have to give a vaccine? What are the optimal intervals? Do we have to change vectors? What is the optimal route of delivery (im vs. sc vs. iv vs. oral etc.)? Which peptides/proteins should we select? How to design a vaccine

that protects from different HCV genotypes? Finally and most importantly, we have to take into account that uncontrolled activation of strong cellular immune responses may also be a double-edged sword because intrahepatic inflammatory activity could potentially be worsened by activation of cellular immune responses.

Promising therapeutic vaccine strategies are currently already explored in phase II trials in patients with chronic hepatitis C infection. These studies will give important insights in the safety and efficacy of peptide-based vaccines for chronic hepatitis C. They might also contribute to our understanding of the immunopathogenesis of the disease. We are now on the bridge to develop new treatment options for hepatitis C that are might be more specific than interferons and associated with less side effects.

Development of a Novel Cell Culture-Derived Influenza Vaccine

N. Barrett

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Influenza viruses for vaccine production are currently produced in embryonated hens' eggs. This conventional standard methodology is extremely cumbersome; it requires a huge amount of eggs and an extensive purification to reduce the amount of contaminating egg proteins and to minimize the risk of allergies against egg albumin. The shortage of eggs in a pandemic situation, the selection of egg-adapted variants and the presence of adventitious viruses has emphasized the necessity for production of influenza vaccines on a well characterized stable cell line. We have developed an alternative cell culture system, which allows rapid production of large volumes of vaccine. The WHO approved Vero cell line was used in serum free culture to grow a multitude of influenza strains to high titre. This system has been scaled-up for vaccine production in a 1200 litre fermenter with antigen yields comparable to the conventional embryonated egg technology. The development of a rapid and efficient purification scheme resulted in a safe, high purity vaccine, which was at least as immunogenic as conventional egg-derived vaccines in a mouse model. Clinical trials with more than 2500 volunteers have demonstrated that the Vero cell derived influenza vaccine is well tolerated, safe and highly immunogenic in humans fulfilling all European CPMP (Committee for proprietary medicinal products) criteria for safety and efficacy of influenza vaccines. This is the first influenza vaccine produced in an industrial scale in tissue culture, which is completely free from egg proteins, antibiotics and thiomersal, thus minimizing the risk of allergic reactions.

Respiratory Vaccines: Viruses and Pneumcoccus

E. Tuomanen

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Respiratory infections constitute a major burden of disease in children and adults. The Children's Infection Defense Center has a programmatic mission to construct multi-component pediatric vaccines against respiratory viruses and bacteria, including parainfluenza (hPIV), respiratory syncytial virus (RSV), influenza and pneumococcus. New reverse genetic techniques, pioneered for influenza, allow complete construction of live viruses and represent a significant new tool for vaccine design. Different hemagglutinins and neuraminidases from highly pathogenic influenza viruses can be combined with structural genes from nonpathogenic variants to generate avian and human vaccines. This will represent the first completely genetically engineered live virus vaccine.

Effective vaccines do not exist for hPIV type 1, 2 or 3 or RSV. To address these challenges, Sendai virus (SeV) is being tested as a xenogenic vaccine for the closely related hPIV-1 and recombinant SeV is being used as a vaccine vector for additional paramyxovirus and RSV antigens. Reverse genetic techniques generate full-length viral cDNA and subsequent infectious particles from the SeV single stranded RNA genome. Recombinants include individual SeV expressing the envelope proteins HN and F of hPIV-1 and 3, and the G and F proteins of RSV. This approach is currently in Phase II testing.

Antigens expressed in these systems can include those from bacteria. A deficit in current pneumococcal conjugate vaccines is the poor protection against mucosal disease events such as pneumonia and otitis. Two antigens critical to mucosal adherence and invasion, Choline binding proteins A and G, have been identified and characterized as vaccines in animal models. We hypothesized that protection even from mucosal infection and colonization is both desirable and achievable for this common community acquired pathogen.

Recognition of identical promiscuous determinants by T cells from different mammalian species and the use of bioinformatics to predict bovine T cell epitopes

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Bioinformatics tools have the potential to accelerate research into the design of vaccines and diagnostic tests by exploiting genome sequences. The aim of this study was to assess whether in silico analysis could be combined with in vitro screening methods to rapidly identify peptides that are immunogenic during Mycobacterium bovis infection of cattle. In the first instance the M. bovis derived protein ESAT-6 was used as a model antigen to describe peptides containing T cell epitopes that were frequently recognised across mammalian species including natural hosts for tuberculosis (humans, cattle) and small animal models of tuberculosis (mice, guinea pigs). Having demonstrated that some peptides could be recognised by T cells from a number of TB infected hosts, we tested whether a virtual matrices-based human prediction programme (ProPred) could identify peptides that were recognised by T cells from M. bovis infected cattle. In this study, 73 % of the experimentally defined peptides from ten M. bovis antigens that were recognised by bovine T cells contained motifs predicted by ProPred. Finally, in validating this observation, we showed that 3/5 peptides from the mycobacterial antigen Rv3019c that were predicted to contain HLA-DR restricted epitopes were recognised by T cells from M. bovis infected cattle. The results obtained in this study support the approach of using bioinformatics to increase the efficiency of epitope screening and selection. Peptides identified in this study are now being tested in cattle as a peptide subunit vaccine.

A Candidate for a Malaria Vaccine: The Merozoite Surface Protein 1 of Plasmodium falciparum

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The MSP-1 complex is the major protein component at the surface of merozoites, the infectious form of blood stage malaria parasites. Studies in animal models as well as sero-epidemiological analyses support the view of MSP-1 of P.falciparum being a promising candidate for a vaccine against the most severe form of malaria in humans. The MSP-1 complex originates from a 190 kDa GPI-anchored precursor, which is proteolytically processed into several fragments which, however, remain non-covalently complexed at the parasite's surface and which interact with a number of other surface proteins. While the C-terminal 10 kd portion of MSP-1 has attracted particular attention, there is at present little reason to exclude any portion of the molecule from examination as a potential vaccine, particularly as MSP-1 may participate in several steps of erythrocyte invasion. On the contrary, by considering the entire protein, one rather follows the concept of a multivalent vaccine.

The goal of our experimental vaccine is to elicit a humoral as well as a cellular response against the two prototypic MSP-1D and MSP-1F variants derived from P.falciparum strains 3D7 and FCB1, respectively. Thus, we have developed a process for the large scale production of both 190 kDa proteins from E.coli. GMP production and formulation of both protein preparations is completed and clinical phase I/IIa studies are foreseen within 2003. For eliciting a CTL response directed against the liver stage of the infection, we follow several approaches which include specialized adjuvans as well as recombinant viral vectors carrying the msp-1 genes. Experiments in the mouse model show that both, efficient humoral as well as CTL responses may be elicited by various immunization experiments.

Vector approaches to vaccination against infectious diseases and cancer

J. W. Almond

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Although killed whole organisms and detergent extracted or recombinant derived sub-units have proved successful as vaccines against a number of infectious diseases, this approach has not been universally effective. In cases, where strong cellular based immune effector mechanisms are deemed important, such as in certain viral diseases or cancer, vectors have been developed that provide an endogenous synthesis of the relevant antigen(s) in the vaccinee. Vectored vaccines may also have other advantages such as ease of industrial production, but this may be off-set to some extent by more challenging regulatory requirements. This presentation will focus on two vaccine vectors being developed by Aventis Pasteur and collaborators. The first is the Chimerivax technology based on the Yellow fever virus vaccine 17D vaccine as a backbone/vector for flavivirus antigens, specifically, Dengue fever. This project, which is being carried out in collaboration with Acambis, has progressed to phase 1 clinical trials and is underpinned by a thorough characterisation of candidate recombinant viruses in animal models. These candidates have all of the characteristics of a safe and effective live-attenuated Dengue vaccine.

A second vector approach, termed ALVAC and based on Canarypox, will be described. Here the aim is to stimulate a cytotoxic T-cell responses and against neo-antigens on cancer cells. Vector constructs will be described which incorporate immunostimmulatory molecules in combination with antigens, which are expressed on colo-rectal cancer and melanoma cells.

Cell-based and Molecular Vaccines against Melanoma – Studies in Man and Experimental Animals

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More than 10 years ago, it was reported for the first time that the repeated s.c. injection of cytokine (e.g., IL-2, GM-CSF) gene-transfected, but not of irradiated, wild type cancer cells into experimental animals results in a state of cancer-specific immunity. Using the murine M3 model of human melanoma, we found this immunity to be protective in both the prophylactic and therapeutic situation. While it was originally assumed that cytokine-secreting cancer cells are directly involved in T cell activation, ample evidence now exists that this is not the case. It rather appears that, once administered, genetically modified cancer cells are subject to attack by NK cells elicited by the vaccine itself. Apoptotic and/or fragmented cancer cells thus generated are then taken up by host antigen-presenting cells, presumably dendritic cells (DC) which, when appropriately activated, have the unique capacity of stimulating productive responses in naïve, resting T cells. It is now quite clear that tumor-associated antigens (TAA) expressed by the cancer cell remnants can be channeled into the MHC I pathway of DC and be displayed on their surface for successful presentation. This finding, termed cross-presentation, provided the rational basis for the development of allogeneic cancer cell vaccines. Such vaccines can be generated much faster than autologous vaccines, are easier to standardize and, thus, much better prone for clinical use. The major drawback of this type of vaccine is its antigenic diversity, making it difficult to measure and adequately monitor the cancer-specific immune response.

With the steadily growing number of molecularly characterized TAA, the development of cancer vaccines with defined antigenic specificity is now feasible. As the application of the TAA/TAA peptide alone bears the danger of inducing antigen-specific unresponsiveness, adjuvants are employed which skew the lymphocytic response into a Th1/Tc1 direction. TAA-encoding naked plasmid DNA, when injected intra- or subcutaneously, is a very powerful immunogen, especially when containing large numbers of unmethylated CpG motifs. We and others have shown that such vaccines target and activate cutaneous DC allowing them to migrate to the draining lymph node and to elicit a tumoricidal CD8+ T cell response. A somewhat different situation occurs when TAA are coadministered with cationic poly-amino acids (cpaa). Using the model antigen -galactosidase, we found that a cpaa/ -gal vaccine was significantly more potent in protecting mice against the growth of -gal-expressing RENCA cells than the protein alone. The protective effect required CD8+, but neither CD4+ T cells nor -gal-specific antibodies. CD8 cell priming occurs in a CD4 T cell-independent fashion, takes place within the draining lymph nodes and is accomplished by day 5 after vaccination. Given the fact that 36-48 hours are needed by cutaneous DC for their journey from the skin to the draining node, we were surprised to find that ablation of the injection sites as early as 1.5. hours after cpaa/ -gal administration still led to protection in a large number of animals. This indicates that certain protein antigens injected intradermally in the context of polycations are quickly transported to the draining nodes where they apparently target immunostimulatory resident DC.

Within the past few years, we have learned a great deal about the phenotype of cutaneous and non-cutaneous DC as well as the factors governing their differentiation from their precursors, their migration and their functional maturation. This knowledge should help us to better target TAA to DC at different anatomical sites, to facilitate their uptake, processing and presentation and, thus, to develop more efficacious DC-based cancer vaccines.

Status of Prophylactic Vaccines Against Cervical Cancer

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Human papillomaviruses (HPVs) infect cutaneous, genital and respiratory epithelia in a tissue-specific manner. Infection with HPVs is widespread throughout the world, and viral infection is closely associated with both benign and malignant lesions. The overall percentage of HPV infection (either current or previously encountered) in a population could be as high as 75%. There are now over 100 HPV types described; from a public health standpoint however, only a small number of these different HPV types cause the majority of clinically important diseases. HPV16 and 18 are strongly associated with high-grade anogenital lesions and invasive cancers and are found in ~70% of all cervical squamous cell carcinomas and ~90% of adenocarcinomas. The causal link of HPV and cervical cancer has been clearly established both from population based studies as well as animal models. Despite the existence of good screening programs for precursor lesions of cervical cancer in the US, there are still ~14,000 cases diagnosed each year and ~5,000 women will die from the disease and screening is expensive. In developing countries where access to routine cervical cytological screening is nonexistent or difficult, cervical cancer is the most common malignancy with an estimate of ~500,000 new cases and ~200,000 associated deaths annually. Therefore, an effective and safe prophylactic vaccine would be highly desirable. Approaches to vaccine design and data from early phase prophylactic clinical trials will be discussed.

Therapeutic vaccination against epithelial cancers with surrogate antigen vaccines: preclinical and clinical results

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Appropriate monoclonal antibodies for passive cancer immunotherapy, after years of mostly unsuccessful work, now finally have proven clinical efficacy and thereby opened a new era in the immunologic treatment of malignancies. An important next step is the development of broadly applicable cancer vaccines that induce therapeutically useful immune responses in the patient. Several groups are pursuing different approaches in this regard and a variety of clinical trials are being conducted.

In an attempt to develop novel therapeutic vaccines against cancers of epithelial origin, we have chosen antibody-based strategies. IGN101 is a candidate cancer vaccine targeting the tumor associated epithelial cell adhesion molecule Ep-CAM. IGN101 consists of a murine monoclonal antibody used as immunogenic protein antigen. Due to certain epitopes of this antibody (mimotopes) that mimic epitopes of Ep-CAM, s.c injections of low amounts of the antibody adsorbed on aluminum hydroxide elicit antibodies against Ep-CAM. It is assumed that this immune response may destroy disseminated tumor cells that cause the development of metastases, thereby improving survival.

The preclinical pharmacology and safety profile of IGN101 was assessed by vaccination trials in Rhesus monkeys and a careful analysis of the induced immune responses. In Phase I and II clinical trials in cancer patients, lack of side effects of repeated vaccinations except transient mild erythema at the injection site was demonstrated. A strong secondary IgG immune response with memory, indicating T-cell help, was detected in all patients. As consistently shown by sequential affinity chromatography, a fraction of the antibodies induced by vaccination with IGN101 bound to recombinant human Ep-CAM. A significant reduction of the number of Ep-CAM positive tumor cells in peripheral blood was found after a vaccination course of four injections. Surprisingly, as demonstrated in a Phase II trial, concomitantly administered standard chemotherapies did almost not negatively influence the induction of a specific humoral immune response by IGN101 vaccination.

IGN301 is a candidate cancer vaccine based on a murine anti-idiotypic antibody used as vaccine antigen that mimics the Lewis Y carbohydrate antigen (LeY). LeY is frequently expressed on epithelial cancers. As shown in Rhesus monkeys, vaccination with IGN301 induces a strong secondary cytolytic IgG immune response against LeY positive tumor cells. A 13-week toxicity trial in Rhesus monkeys did not reveal any side effects or toxicities. In a Phase I trial in cancer patients lack of side effects was confirmed and specific IgG immune responses were found.

Clinical efficacy of these candidate cancer vaccines will have to be assessed in Phase II/III trials aiming at demonstration of survival prolongation. With IGN101, double-blind placebo controlled trials with survival parameters as primary endpoints already have been initiated.

E1 vaccination in chronic HCV patients: Well-Tolerated and Possible Halting of Fibrosis Progression in Patients

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PHASE I: Three IM injections of 20 µg of E1 at three-weekly intervals were given to 20 healthy volunteers. All mounted anti-E1 antibodies, and 18 raised a strong and specific cellular immune response upon E1/alum vaccination. Such immune responses have only occasionally been encountered in patients with chronic hepatitis C. Mild and transient side-effects at the site of injection were present in 5 volunteers (and were the only adverse events related to the study treatment).

PHASE IIa: Thirty-five patients with chronic hepatitis C infected with HCV genotype 1 were randomized to receive 20 µg of recombinant E1 (n = 26) or placebo (n = 9) IM at weeks 0, 4, 8, 12, and 24. Thirty-four then re-enrolled in an open-label extension, receiving E1 at weeks 50, 53, 56, 59, 62, and 65. Twenty-four patients (12 male, 12 female; mean age, 52 years; 18 interferon-based treatment failures; mean baseline ALT, 118 U/L) were biopsied pre-baseline and after two courses of E1, approximately 17 months later. Liver histology was scored blinded by two pathologists.

Conversion from a negative to a strong E1-specific T-help response was observed in the vast majority of the patients. The levels of anti-E1 antibodies increased on average three- to fourfold after the second course of E1 injections. In the E1-treated patients, serum ALT decreased significantly from baseline from week 16 onwards. Blind scoring of the liver histology using the Ishak and Metavir systems revealed that liver fibrosis had improved by one point or more in 38% of the patients. The increase in anti-E1 antibody levels correlated with the decrease in total Ishak score, the relative decrease in Ishak fibrosis scores and in ALT (all $p \leq 0.01$). Liver histology improvement following therapeutic vaccination was achieved, while levels of HCV 5’NCR-RNA remained unchanged in the blood. In contrast, the quantity of E2 protein present in the liver was markedly decreased or became negative in 46% of patients.

Further patient follow-up during maintenance therapeutic vaccinations will examine the disease-modifying effects over the long-term. A new study is starting to confirm these new and promising findings.

Virus-like particles: Combining Innate and Adaptive Immunity for Effective Vaccination

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The immune system has been educated to distinguish between non-infectious self and infectious non-self. Hence, it mounts strong immune responses upon microbial infection but usually fails to respond to self molecules. Understanding the mechanisms of this self/non-self discrimination by the immune system is critical for our ability to rationally design vaccines. In recent years, it has become evident that several factors in addition to B and T cell tolerance contribute to self/non-self discrimination. As discussed in the following, these parameters include order and repetitiveness of antigenic epitopes as well as activation of the innate immune system.

Most viruses consist of few proteins only with structural restrictions impinged on them. In fact, using these few proteins, viruses are forced to generate a quasi-crystalline, highly repetitive surface. Such highly repetitive antigens efficiently cross-link B cell receptors, a process which generates a strong activation signal in B cells. In contrast, self antigens are usually not highly organized, in particular not those accessible to B cells. Thus, B cells use antigen organization as a marker for infectious non-self. Vaccines based on virus like particles (VLPs) harness this basic phenomenon, since VLPs exhibit surfaces as repetitive and organized as those of viruses. Consequently, similar to viruses, VLPs are able to trigger strong B cell responses in the absence of adjuvants. Conjugating antigens to VLPs renders them as repetitive as the underlying VLP surface. Antigens coupled to VLPs therefore become highly immunogenic in many species, including humans.

Although VLPs trigger strong antibody responses in the absence of adjuvants, T cell responses are much weaker, in particular if highly purified antigen preparations are used. Thus, in contrast to B cell responses, which seem to be comparable to those induced by whole viruses, T cell responses induced by VLPs are inefficient. This is rather surprising, since VLPs are only processed by professional APCs such as dendritic cells and macrophages and not by B or T cells, in principle favoring induction of protective immunity. However, purified VLPs fail to stimulate innate immunity and this seems to be responsible for the poor T cell responses. Indeed, if VLPs are loaded with bacterial DNA, which stimulate activation of dendritic cells through toll-like receptor 9, fulminant T cell responses may be induced that surpass CTL responses induced by recombinant vaccinia viruses and are able to eliminate established tumors in mice. Thus, VLPs filled with CpGs induce B and T cell responses as efficient as live viruses, indicating that the combination of high epitope density with stimulation of innate immunity are the key parameters that distinguish live viruses from their isolated proteins.

Recombinant live oral vaccines against bacterial enteric pathogens: the way through regulation

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Live oral vaccines based on attenuated pathogens present the best stimulation of the mucosal immune system and possess distinct advantages compared to parenteral vaccines. However, despite the promising outlook of recombinant DNA technology; impressive achievements in the understanding of pathogenicity mechanisms; and innumerable research projects aimed at the development of safe and effective attenuated strains as vaccines or vectors, few of the latter have entered clinical trials. For the time being only a single recombinant live oral vaccine has been licensed for immunisation of humans worldwide.

The vaccine strain in question, CVD 103-HgR, was tailor-made from a wild type Vibrio cholerae. It harbours a site-specific deletion in the locus encoding cholera toxin and, for the sake of strain identification, a genetic locus encoding mercury resistance was inserted into the haemolysin genetic determinant. The strain was extensively characterised both genetically and phenotypically. It has been shown to be very safe and highly immunogenic in humans and to confer protection against wild type challenge after administration of a single vaccine dose.

The presentation will primarily focus on the issues pertaining to the acceptance of recombinant live oral vaccines by regulatory authorities. Based on the expertise gained in the registration of cholera vaccine strain CVD 103-HgR, issues such as (i) genetic structure; (ii) genetic stability; (iii) residual pathogenicity; (iv) potential for gene transfer (as donor and/or recipient); (v) capacity to survive, establish and disseminate in the environment, etc., will be discussed.

Delivering T-cell Immunity with Heat Shock Proteins

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In order for tumor or virus-infected cells to be efficiently recognized by the T-cell system in vivo, antigens derived from these dying cells need to be productively transferred to professional antigen presenting cells (such as dendritic cells) for processing and T-cell presentation. One of the mechanisms implicated in this phenomenon (known as immunological cross-priming) involves the transfer of antigen in association with heat shock proteins (HSPs). Accordingly, several vaccine strategies involving purified HSP-antigen complexes have been developed to take advantage of this natural pathway for eliciting T-cell immunity. These will be reviewed with a special emphasis on their utilization to induce CD8 cytotoxic T-lymphocyte responses. HSPs are known to bind to specific receptors on professional APCs , enabling them to mediate two specific events required to launch a T-cell immune response. First they facilitate entry of the exogenous HSP-antigen complexes into the endogenous antigen processing pathway, a step required for the loading of peptide antigen onto class I MHC molecules for presentation to CD8 T-cells. Secondly, cell surface binding of the HSP complex triggers maturation/differentiation signals which permit the APC to develop its full presentation potential. This combination of antigen delivery and APC activation (introducing the “stranger” in the context of “danger”) bridges the innate and adaptive immunity imperatives, thereby creating within a single molecular complex the potential to induce potent T-cell immune responses. Several such HSP-based immunization strategies have now been advanced into the clinic, addressing both cancer and chronic viral indications. These will be reviewed with an emphasis on understanding how the creation of high affinity complexes between HSP70 and defined synthetic peptide antigens affords specific advantages in the design of such therapeutic vaccines.

Transcutaneous Immunization and Immunostimulant Strategies: Capitalizing on the Immunocompetence of the Skin

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The skin, especially the epidermal layer, is an accessible and competent immune environment that is an attractive target for vaccine delivery. Recent work focused on vaccine delivery into the skin using a patch or similar means has established skin delivery as a new vaccine paradigm. In particular, we have shown that the use of adjuvants in the context of the skin results in far greater immune responses compared to antigens alone. Heat labile enterotoxin from E. coli, LT, has been found to be particularly suitable as an adjuvant for skin delivery as it is potent, can be safely used on the skin, and is available at commercial scale. LT has been formulated in dry, adhesive patches that are e readily manufactured, stable and which deliver effective and commercially viable amounts of material. The formulated patch may be combined with antigens in a patch (Transcutaneous immunization or TCI) to elicit immune responses to the coadministered antigen. LT may also be delivered to the skin separately from injected antigens to enhance the immune response, as an immunostimulant (IS) patch. The IS patch can be simply applied in place of a bandaid after an injection and offers the ability to simply add adjuvant to licensed vaccines or late stage vaccines where immunogenicity is lacking. Both TCI and IS patches elicit potent T cell responses, high levels of IFNg, as well as antibody responses and can be used to stimulate regional immunity. These applications of the original observation that adjuvants and antigens can readily be delivered into the skin are currently supporting Phase 2 trials.

Vaccination and immunological memory

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Vaccination acts by inducing immunological memory that protects from subsequent encounters with pathogens or toxins. Primed individuals not only can mount secondary immune responses that are more rapid and effective than primary responses, but also maintain, in the absence of further boosting, a certain level of effector T cells and antibodies for a lifetime. These aspects of immunological memory have a distinct cellular basis. Recall responses are mediated in secondary lymphoid organs by central memory T cells and memory B cells, while immediate protection is mediated in peripheral tissues by effector memory T cells and by antibodies produced by long-lived plasma cells. I will review the experimental evidence supporting a "stem cell model" of immunological memory. Central memory T cells and memory B cells are intermediates of a progressive differentiation process, which have acquired the capacity to proliferate and differentiate in response to polyclonal stimuli such as cytokines, microbial products or bystander T cell help. While self renewing, central memory T cells and memory B cells continuously spill out effector T cells and plasma cells, thus replenishing those that turn over. I will describe in details the mechanisms that sustain serum antibody levels following vaccination and discuss the implications of these findings for vaccine design.

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Poxvirus Vectors with and without DNA Priming

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Vaccinia virus, a member of the poxvirus family, was successfully used as a live vaccine for the eradication of smallpox. Subsequently, vaccinia virus and avian poxviruses were developed as expression vectors and shown to protectively immunize animals against a variety of other infectious diseases and cancer. Because of the side effects encountered during smallpox vaccination, a number of attenuated strains of vaccinia virus were derived. One of these, derived by A. Mayr and co-workers in Munich and known as Modified Vaccinia Virus Ankara, has a severe host restriction to chick embryo fibroblasts but can achieve high levels of viral and recombinant protein expression in a one-cycle infection of humans and other mammalian cells. Moreover, the immune response in rodents and non-human primates can be as good or superior to that achieved with replicating strains of vaccinia virus, possibly due to deletion of viral immune defense genes. There has been renewed interest in the use of MVA as a smallpox vaccine and MVA recombinants have been shown to protect non-human primates against disease caused by parainfluenza virus 3, measles virus, simian immunodeficiency virus, and simian human immunodeficiency virus. Priming with DNA or other vectors followed by boosting with an MVA recombinant significantly increased the immune response over that obtained with either vector alone. Pre-clinical and clinical studies to determine the safety and immunogenicity of MVA-based smallpox and recombinant HIV vaccines are in progress.

Bacteria mediated DNA transfer for gene therapy and vaccination

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Bacteria have been shown to be able to transfer eukaryotic expression plasmids into the nucleus of host cells. Depending on the type of bacteria, two major pathways became apparent. Bacteria might escape from the phagocytic vacuole into the cytosol of the host and deliver their plasmid load upon lysis due to metabolic attenuation, autolysin expression or antibiotic treatment. Alternatively, bacteria might invade the cell but remain in the phagocytic vacuole and after lysis due to metabolic attenuation deliver their plasmids via an unknown pathway into the nucleus of the host cell for expression. A prototype of the latter transfer vehicle is Salmonella typhimurium aroA, which is no longer able to synthesize aromatic amino acids and thus is able to only survive in vivo to a limited extend.

Oral administration of such bacteria carrying eukaryotic expression plasmids that encode various antigens has been shown to induce antigen specific CD8 and CD4 T cells as well as antibodies. A single administration was already sufficient to induce a good immune response. In addition efficient memory responses were induced under such conditions. Plasmid transfer to the host was essential for the induction of these immune reactions.

Analysis of the kinetic and the cells that are involved in this oral vaccination revealed that antigen is detected very rapidly after administration and remains detectable for more then a month, thus explaining the efficient induction of an immune response after a single administration. Antigen is found to be expressed in macrophages but also in dendritic cells. However, cross-presentation via dendritic cells seems to be an important aspect of Salmonella mediated oral DNA vaccination.

One problem that was encountered during these experiments was the stability of the plasmid carrier. The original plasmid contained a pUC origin of replication (ori) that leads to a high copy number within the bacterium. This obviously has adverse effects with regard to the in vivo survival of plasmid carrying Salmonella. Therefore, we exchanged the ori of the original plasmid with ori`s leading to a low copy number of the expression plasmid in the bacteria carrier. This change leads to a dramatically improved survival of the plasmid carrying bacteria in vivo and an enhanced immune response. Such manipulations now allow the introduction of plasmids carrying immune modulatory genes along with the antigen encoding plasmid since the use of compatible ori`s enable the bacteria to carry different plasmids.

Delivery systems for Vaccines and Adjuvants

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Although DNA vaccines have generated significant immune responses in laboratory animal studies, including protective immunity in infectious and non-infectious disease models and in neonates, human clinical studies of DNA vaccines have yielded weak immune responses. In general, DNA vaccines are most effective at inducing CTL responses, while helper T cell and antibody responses are more modest. Therefore, for DNA vaccines to be fully effective in humans, enabling technologies must be developed in order to increase the potency of these responses. To increase the potency of DNA vaccines we have focused on two main areas: i) form of plasmid (including alphavirus replicon DNA), and ii) improved DNA delivery technologies (including physical and particle-mediated delivery). With regard to the latter, two approaches were taken, based on areas we identified as barriers to efficient in vivo DNA delivery. First, electroporation was used to facilitate distribution of DNA within the injected tissue and uptake of DNA by muscle cells. Second, a cationic microparticle (PLG) with adsorbed DNA was used as a carrier to target APCs. These systems were shown to be up to 1000-fold more potent for induction of both antibody and T cell responses in animal species ranging from mice to non-human primates. In addition, these delivery systems are effective for and complementary with the use of adjuvants to further potentiate antigen-specific immune responses. Therefore, these DNA vaccine technologies can serve as potent inducers of immune responses in animal models and hold promise for use in humans.

Reverse vaccinology, a genome-based approach to vaccine development

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Since the discovery that microbes cause infectious diseases and that vaccination can be used to prevent them, the first task in vaccine development has always been to grow the pathogen in vitro order to develop live-attenuate or killed vaccines or to purify antigens to be used as subunit vaccines. The DNA sequence of the whole genomes of microorganisms allowed for the first time to tackle vaccine development starting from the computer prediction of protective antigens and to obtain vaccine candidates without the need of growing microorganisms. In order to underline the different path to vaccine discovery that has been made possible by the genomic sequencing, this new method has been named "reverse vaccinology".

Reverse vaccinology is not only an alternative method to discover antigens, but also a powerful tool to tackle development of those vaccines where the conventional technologies had failed. The first example of a genome-based vaccine development has been serogroup B meningococcus. In this case, forty years of vaccine research using the conventional approaches had not provided vaccines able to immunize against all strains causing the disease. The availability of the genomic sequence made available at once all potential antigens encoded by the bacterium. 600 novel potential antigens were identified by computer prediction, expressed as recombinant proteins in Escherichia coli and tested as vaccines within 18 months. 29 novel antigens were found to be good vaccine candidates. The best among the novel antigens have been used in subsequent studies to design an universal vaccine to be tested in clinical trials. The same approach used for meningococcus B is now being applied to several microorganisms and is likely to lead to the development of many novel vaccines.

Identification of the “antigenome” - a novel tool for the design and development of subunit vaccines against bacterial pathogens

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The human immune system is capable to identify and eradicate pathogens and pathogen-infected cells, based on the recognition of antigenic structures of an intruding microbe, defined as antigens. In order to identify novel vaccine candidates for a number of bacterial pathogens, human sera were selected from patients and healthy individuals with high levels of functional anti-bacterial antibodies. Inspired by the known shot-gun strategy used for sequencing whole genomes, bacterial surface proteins were employed as display platforms to present at the surface of E. coli cells frame-selected genomic peptide libraries. This approach, relying on human sera, which most likely contain antibodies protecting against disease, and genomic-based peptide libraries encoding possibly all antigenic sequences of a pathogen, identified a comprehensive set of approximately 100 antigenic proteins, which we refer to as the “antigenome” of a human pathogen. To further select from the “antigenome” the most promising candidates for vaccine development, a coherent and rapid procedure was established. The most promising antigens must be conserved among clinical isolates, be expressed in individuals upon encounter with the pathogen, surface displayed or involved in vital virulence functions and targeted by functional antibodies. The validation procedure addresses these functions and allows the selection of only a small number of antigenic proteins that need to be tested in animal models. The value of this approach is underlined by the successful identification of most published protective antigens from several human pathogens, including S. aureus, S. pyogenes, S. pneumoniae and S. agalactiae.

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Novel adjuvants for the induction of cellular and humoral immune responses

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Vaccines have saved more lives than any other medical intervention. The vast majority of vaccines used in humans induce antibody responses. Whilst useful for a number of vaccine preventable illnesses, T cells rather than antibody responses are required e.g. to develop vaccines against most viral infections and for therapeutic vaccines. This cannot be achieved with adjuvants currently on the market: Aluminium hydroxide, the adjuvant used in almost all adjuvanted vaccines does not induce T cells. In addition, several vaccines need improving, as they do not optimally function. Thus, the search for and the development of novel T cells adjuvants is warranted.

Here, we present two very promising novel adjuvants: IC30 (poly-L-Arginine), which is a potent enhancer of T cell responses when combined with peptide antigens. IC30 is currently tested in several clinical trials. IC31 is a compound that strongly induces Th1 type immune responses. Which in our hands is superior to other adjuvant technologies. Furthermore, IC 31 does not induce the potentially harmful systemic production of pro-inflammatory cytokines (e.g. TNF- , IL-6). Both compounds have a favourable toxicological profile and can be manufactured according to regulatory requirements at low cost.

Data demonstrating the efficacy of IC30and IC31 and their mechanisms of action will be presented.

Ultra-sensitive DNA and protein biochips driving fluorophores via a nano-cluster layer

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Nowadays DNA hybridization micro-arrays are widely used to quantify DNA as well as investigate DNA-DNA, DNA-RNA or DNA-protein interactions. To boost fluorescence signals on chip we developed a novel set up amplifying standard fluorophores such as Cy,3, Cy5 or Rhodamine via a nano-cluster assembly (SEF).

A fundamental problem of biochip technology is the poor signal compared to other established methods such as membrane blots or radionucleotide techniques. Metal-nanoclusters (nano-sized metal particles, ~5-200 nm) or nano-films (metal thin films of ~ 10 - 200 nm in thickness) have the potential to amplify fluorescence and thus to enhanced the signal of labelled biomolecules on chips surfaces. Fluorescent molecules are bound at a certain distance to a layer of metal-clusters driven in structured optical resonance (often referred to as 'resonant layer'), resulting in enhanced absorption and emission of the fluorophore within the resonant field. Surface-enhanced fluorescence (SEF) enables to considerably improve the signal intensity deriving from a novel class of bio-nano-devices Using these resonant assemblies, we recently obtained enhancement factors of up to ~200 and proved the suitability of the devices to set up ultra-sensitive biochips.

Oligo-arrays as well as phage display based protein SEF-biochips are fully compatible to any standard slide format fluorescence scanner.

Identification of novel Mycobacterium tuberculosis antigens with potential as diagnostic reagents or sub-unit vaccine candidates by comparative genomics

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Vaccination with Bacille Calmette-Guérin (BCG), the only currently available vaccine, results in tuberculin PPD-sensitivity and has shown variable vaccine efficacies in cattle. Thus, identification of more specific reagents to distinguish between vaccination and infection as well as the identification of sub-unit vaccine candidates for improved TB vaccines are research priorities in the effort to develop novel TB vaccination strategies for cattle. In the present report we applied comparative genomics to identify M. bovis/M. tuberculosis antigens whose genes had been deleted in BCG. In total thirteen open reading frames (ORF) from the RD1, RD2, and RD14 regions of the M. tuberculosis genome were selected. Pools of overlapping peptides spanning these ORFs were tested in M. bovis infected (n=22), BCG vaccinated (n=6), and un-vaccinated control cattle (n=10). All were recognized in infected cattle with responder frequencies varying between 16 and 86%. In particular, six antigens showed promise as diagnostic antigens (Rv1983, Rv1986, Rv3872, Rv3873, Rv3878 and Rv3879c), and two more as potential vaccine candidates (Rv1979c and Rv1769).

Combined Formulation of Hepatitis B Virus Antigens as a Nasal Therapeutic Vaccine Cancidate

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The purpose of this study is the evaluation of the humoral immunity raised with a combined formulation of Hepatitis B Virus antigens.

One of the main mechanisms involved in HBV chronicity is the tolerance induction to the HBV antigens.

We have immunized Balb/c mice with different formulations containing HBsAg and HBcAg through the nasal route in a total volume of 50 µL per mice. The IgA, IgG, IgG1 and IgG2a titers in sera and vaginal washes were determined by ELISA. The interaction between HBsAg and HBcAg on HBsAg immunogenicity was carried out using as control adjuvants cholera toxin and acemannan.

We observed a strong adjuvant effect of HBcAg on HBsAg immunogenicity in serum and vaginal secretions, similar in intensity to CT and acemannan. We also achieved a higher and significant increase in the rate IgG2a/IgG1 and CTL response against HBsAg for the group immunized with HBcAg and HBsAg.

We conclude that the inoculation of the soluble formulations of HBsAg and HBcAg enhanced the immunogenicity of HBsAg in serum and mucosal secretions, and modulated the IgG subclass pattern to the Th1-like pattern.

Optical Nanocluster Plasmon-Sensors as Transducers for Bioaffinity Interactions

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The choice of metal clusters as signal transducers of molecular binding events is based on their up to 1000 times higher extinction coefficients compared to conjugated chromophores and superior stability due to the lack of photo – bleaching. Using cluster based assays it is possible to visualize the binding of biomolecules at a given surface by a bound layer of ligand-modified metal clusters. A similar direct approach of detection is impossible with chromophores without using an additional amplification by enzymes e.g. employed in enzyme linked immuno assays.

The chip is constructed by depositing multiple nanoscale layers on the surface of a CD-ROM or DVD like substrate. The high spatial resolution together with combinatorial chemistry on nano-particles can be achieved by photo patterning of the sensor surface. The signal can be read out on scanners, CD-ROM-based devices or by means of video-microscopy.

Based on this sensor setup either a chemically reactive distance layer or biochemical linker molecules sensitive to the analyte were employed.

While established methods require a maximum distance of the molecules in the range of only a few nanometres, our system allows detection of binding events from twenty to several hundred nm. Concentrations in the femtomol / mm^2 or pmol/l range have been detected.

The focus of our development was to provide an optical microarray, which allowed detection of analytes such as DNA, RNA, proteins as well as drug candidates. The invention enabled us to replace conventional binding assays overcoming various technological limits as there are multiple incubation steps, harmful reagents and spatial resolution and allows rapid integration into existing drug screening programs.

Epibase, a new platform for fast and accurate identification of T-cell epitopes

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We have developed a first principles computational method to identify T-cell epitopes for any type of MHC class I molecule. The method, which is based on sound physico-chemical principles, is also applied to MHC class II molecules. Candidate T-cell epitopes are docked on the MHC molecule of choice using a docking procedure that operates at atomic detail. The peptides (typically nonamer peptides) are treated as fully flexible objects. Also the MHC receptor's side chains are considered as flexible allowing these to optimally interact with the binding peptide. The combinatorial complexity of the docking procedure is efficiently handled using a FASTER-based algorithm (Desmet et al., Proteins, 48, 31-43,2002). The docking process has been implemented in our Epibase platform which runs on a LINUX cluster containing 96 CPUs. Typically, for a given protein of interest, all possible 9-mers are docked on a collection of different types of MHC molecules. As a result, each peptide is attributed a score which allows to rank the peptides according to their estimated binding strength. To extend our Epibase platform, we have implemented a robust cell-based binding assay. Applying the Epibase platform to the Hepatitis B surface protein with respect to the MHC class I A2 and A24 molecules, we have identified new CTL epitopes and have demonstrated that the predicted affinities correlate with the measured affinities for these epitopes. Clearly, this validates the Epibase platform and underlines its power to identify novel epitopes that are unlikely to be found with routinely used statistical approaches.

Immune and pathogenic effects of modified vaccinia Ankara and formalin-inactivated vaccines in respiratory syncytial virus infection.

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Development of vaccines for respiratory syncytial virus (RSV) has been greatly constrained by the disastrous outcome of trials of formalin-inactivated vaccines (FI-RSV), which triggered severe disease exacerbation in children. It is now clear that understanding the mechanisms causing disease enhancement is essential to the development of safe RSV vaccines. The immunopathology has been reproduced in mice and allows the comparison of new vaccine candidates with FI-RSV. We used modified vaccinia Ankara (MVA) expressing RSV F or G surface glycoprotein (MVA-F and MVA-G) and show that these vaccines were able to restrict virus replication during subsequent pulmonary RSV infection. MVA-F and MVA-G induced both IgG1 and IgG2a, whereas FI-RSV induced a strong IgG response biased towards IgG1. Higher IL-12 and IL-18 levels in bronchoalveolar lavage fluid as well as fewer IL4, IL-5 and more IFNγ producing cells were detected in the lung of MVA-F or MVA-G-immunized mice when compare with FI-RSV immunization. MVA-RSV vaccination did not cause the striking lung eosinophilia induced by FI-RSV sensitisation. Even so, immunizations of mice with all RSV-specific vaccines caused transient weight loss and respiratory illness upon RSV challenge reminiscent of RSV-specific immunopathology. We conclude therefore that MVA-RSV vector vaccines can induce a qualitatively distinct immune response profile, possibly advantageous in vaccine development. However, additional investigations are necessary in other animal models less prone to immunopathology.

DegP and related genes as stress-markers for E.coli-viability - ultra-sensitive RT- Realtime PCR -

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The study was done to set up an on-line viability test system based on the stress-response of pathogenic E.coli or living cells in general. Fully automated and computerized pathogen detection was achieved via RNA expression monitoring using an high-speed Real-time PCR-system with single molecule sensitivity. The device was developed in a collaboration of SAUR Group - France, the TU Delft (Kluyver Laboratorium – Analytical Biotechnology, Netherlands) and Attophotonics Bioscience, Austria.

The automated viability assay is fully hands-off, using a continuous concentration process of the pathogens from litres sample down to tens of millilitres, cell cracking to DNA/RNA solution and further concentration down to a few hundred microlitres. RT-PCR and Realtime-PCR are done with a robotic system. DegP found from E.coli to human was selectively induced within the device via heat shock to 50°C. Up to 1000 fold induction was achieved from environmental to heat-shock level. It was proven that degP quantification via RT-Real time PCR provides an excellent basis for multi-organism viability detection. This fully automated device will provide a fast and accurate tool to detect harmful pathogens from different sources e.g. drinking water, food cans and various medical issues.

Efficacy of a Novel DNA Vaccine in Rabbits Challenged with Bacillus anthracis Spores, Ames Strain

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Bacillus anthracis, the etiologic agent of anthrax, is a large, gram-positive, rod-shaped, non-motile, facultative anaerobic, spore-forming bacterium that produces disease in humans and animals. Many countries of the world experience periodic natural outbreaks of both animal and human anthrax. More importantly, B. anthracis is capable of forming spores that are highly stable to a wide range of environmental conditions. In an aerosolized form, the spores can cause inhalational anthrax, which is greater than 50% fatal, even with appropriate treatment. For these reasons, B. anthracis is considered a serious bioterrorist threat. It is generally accepted that a major facet of protection against anthrax infection is an effective humoral immune response against the protective antigen (PA), one of the three toxin proteins produced by the infecting microorganism. This study examined the ability of differing regimens of novel DNA vaccines to elicit an immune response to PA and to protect against an aerosol challenge with B. anthracis in the rabbit model.

Rabbits were vaccinated with different regimens of DNA vaccines (Table 1). Vaccinated (15) and naive control (4) rabbits were aerosol challenged with B. anthracis spores, Ames strain, with an average dose of 50 LD50s with a range from 18 to 169 LD50s. Of the five vaccinated rabbits that survived two were immunized intramuscularly (i.m.) with DNA followed with a protein boost and three were immunized subcutaneous (s.q.) with protein. A major factor predicting survival was the ability of the animal to mount a lasting antibody response to PA.

Rabbit sera were collected prior to aerosol challenge and titrated for PA antibodies by indirect ELISA. A probit dose-response model was fitted to the anti-PA antibody titer dose-lethality response data. Model parameters were used to estimate 50 percent and 90 percent protective antibody titers (PT50 and PT90). The relationship between anti-PA antibody titer and protection was statistically significant. The estimated PT50, titer to protect 50 percent of the challenged animals was 242, and the estimated PT90, titer to protect 90 percent of the challenged animals, was 1132.

IL-4 producing CD8+ T cells with a CD62L++(bright) phenotype accumulate in a subgroup of older adults and are associated with the maintenance of intact humoral immunity in old age

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An increased production of proinflammatory cytokines occurs in a high percentage of elderly persons and is associated with an impaired humoral immune response. However, high IL-4 production has also been observed in old age. We now demonstrate an IL-4 producing subpopulation of CD8+ T cells in a subgroup of healthy older adults. This T cell subset is substantial in size and has a characteristic phenotype expressing CD45RO, CD28, CD62L and CD25. IL-4 producing CD8+ T cells produce large amounts of IL-2 but neither IFN- nor perforin and do not have a regulatory suppressive effect on other T cells. In vivo IL-4 producing CD8+ T cells can be stably detected over a year. When put into culture they also have a stable cytokine production pattern but fail to produce perforin even in the presence of IL-12. This special T cell type does not occur under the age of 40 but is present in 36% of the persons of more than 60 years. In this age group IL-4 producing CD8+ T cells are more frequent in persons who are still capable of raising a humoral immune response following immunisation than in others who fail to produce protective antibodies after vaccination. Our results suggest that CD8+ T cells with a CD62L++(bright) phenotype accumulate in a subgroup of older adults. Due to their phenotype, which enables them to migrate into lymphoid tissues and their capacity to produce IL-4 these cells may counterbalance the overproduction of proinflammatory cytokines in old age.

C2B8 treatment of B-cell Chronic Lymphocytic Leukemia Cells promotes cross priming of autologous cytotoxic T-cells by bystander DC

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C2B8 is a chimeric monoclonal antibody (mAb) against the human B-cell restricted cell surface antigen CD20 which is used as an alternative and effective medication for B-cell Non Hodgkin Lymphomas (NHL). Treatment of CD20+ B-NHL cells with C2B8 induces apoptosis. Dendritic cells (DC) have been shown to ingest apoptotic cells and cell debris and to cross-present associated antigens on MHC-class I molecules stimulating potent CTL responses. In our previous work we succeeded to demonstrate evidence for the induction of a lymphoma cell specific cytotoxic T-cell (CTL) response upon lymphoma treatment with C2B8 in a model system: C2B8 treated Daudi cells became apoptotic and were readily phagocytosed by dendritic cells (DC). The immature DC used in these experiments underwent maturation when cocultured with C2B8 treated lymphoma cells and promoted the induction of specific cytotoxic T-cell (CTL) responses (Selenko et al, Leukemia 2001; 15:10). In the present work we have extended our studies and performed autologous ex vivo experiments with lymphoma cells from patients with B-CLL. We were prompted to investigate whether C2B8 treatment of B-CLL cells promotes the induction of an autologous lymphoma cell specific CTL-response via cross presentation by bystander DC. The DC were generated from separated monocytes of the B-CLL patients and were phenotypically and functionally characterized. After treatment with C2B8, Dendritic Cells from 7 out of 10 B-CLL patients could stimulate cytotoxic T-cells to specifically lyse autologous lymphoma cells. Taken together, our results suggest that antibody treatment of lymphoma cells with C2B8 can indeed promote the induction of specific CD8+ CTL activity. However, since the decision whether the C2B8 engaged lymphoma cell becomes an immunogen is hard to predict, it should be considered favour clinical studies using C2B8 in combination with DC activating stimuli such as CD40L of IFNα or IL12 in order to ensure and possibly boost priming conditions.

Mechanism and applications of CpG oligodeoxynucleotides as an adjuvant for infectious diseases and cancer vaccines

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Small oligodeoxynucleotides (ODN) with unmethylated CpG moditfs are able to perfectly mimic the immunostimulatory activity of bacterial DNA and are recognized by the Toll-like receptor 9 (TLR9) molecule. TLR9 is expressed exclusively by human B cells and plasmacytoid dendritic cells (pDC). CpG ODN stimulate B cells to proliferate and secrete a variety of Th1-like cytokines and type I interferons and up-regulate co-stimulatory molecules. Different classes of CpG ODN could be identified that show distinct immunomodulatory profiles.

CpG ODN are extremely effective Th1 adjuvants and have shown therapeutic activity in animal models of infectious and allergic diseases as well as in therapeutic vaccination of established tumors in mice. In human clinical trials a CpG ODN, ODN 7909 appears to induce earlier seroconversion with the production of markedly increased immune responses as an adjuvant or a hepatitis B vaccine.

Virosomes: immunostimulating delivery system with broad applications

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Virosomes, reconstituted influenza virus envelopes of approximately 150 nm in diameter, are composed of the influenza surface glycoproteins haemagglutinin (HA) and neuraminidase (NA) and a mixture of natural and synthetic phospholipids. The virosome production process ensures that the fusogenic properties of the influenza virus HA are maintained, thus facilitating the binding and subsequent endocytosis of the virosome and any adsorbed or incorporated antigen into immunocompetent cells. Without eliciting the migration of inflammatory cells towards the application site and consequently causing few local reactions, the biodegradable virosomes can iniatiate an efficient immune response.

The virosome delivery system – in wide clinical use for a hepatitis A vaccine since 1994 and for an influenza vaccine since 1997 – can present a wide variety of antigens to the immune system, such as bacterial toxins, inactivated viruses, recombinantly produced proteins, synthetic peptides or plasmids. While adsorption to aluminium salts impairs the conformational structure of peptides, the association with virosomes preserves the native status B or T cell epitopes. Thus, in comparison to conventional adjuvants, peptides-based virosomal vaccines have been shown to elicite higher levels of functional antibodies. By positioning the antigen either on the surface or inside the virosome, the immune system can be directed preferentially towards a Th2 or Th1 type response.

Besides the use as delivery and adjuvant system for prophylactic vaccines, the virosome technology holds promise for additional applications, such as therapeutic vaccination, drug delivery, and cell targeting for treatment of tumors.

Author Index

JC. Aguilar, 32
R. Ahmed, 7
S. Akira, 4
Y. Alguel, 30
J. W. Almond, 14
M. F. Bachmann, 19
B. H. Barber, 21
R. Barnewall, 37
N. Barrett, 10
K. Boulez, 34
N. Boutonnet, 34
H. Bujard, 13
M. Buschle, 29
B. Castelein, 34
K. De Clercq, 34
P. J. Cockle, 31
E. Depla, 18
J. Desmet, 34
D. Doornbosch, 36
Ch. Epp, 13
J. Estep, 37
A. von Gabain, 1, 28
D. Galloway, 37
A. Gandhum, 30
G. Glenn, 22
S. V. Gordon, 31
M. Gréco, 2
B. Grubeck-Loebenstein, 38
G. Guillén, 32
J. Haglmüller, 33
M. Hanner, 28
T. Henics, 28
R. G. Hewinson, 12, 31
I. Idler, 13
E. Jäger, 5
U. Jäger, 39
K. U. Jansen, 16
B. Jenewein, 38
M. Jun, 13
Ch. Kauth, 13
R. Kiessling, 6
W. Knapp, 39
A. Knuth, 5
A. Lanzavecchia, 23
I. Lasters, 34
A. Liner, 37
Y. Lobaina, 32
H. Loibner, 17
R. Lutz, 13
O. Majdic, 39
M. P. Manns, 9
A. Mateczun, 37
V. Matyushin, 30
Ch. Mayer, 30, 33
G. Meersseman, 34
A. Meinke, 28
D. B. Minh, 28
B. Moss, 24
V. Muzio, 32
E. Nagy, 28
St. Normark, 3
W. Olszewska, 35

P. J.M. Openshaw, 8, 35
M. Overeijnder, 36
E. Pentón, 32
D. Pichardo, 32
F. Pittner, 33
J. Pletinckx, 34
B. Price, 37
R. Rappuoli, 27
H. Rauter, 36
P. Robatscher, 38
D. Robinson, 37
Th. Schalkhammer, 30, 33, 36
R. Schroeder, 33
S. Schwaiger, 38
N. Selenko-Gebauer, 39
C. Sillaber, 39
T. Stegmann, 34
N. Stich, 30
G. Stingl, 15
J. Stoeckl, 39
Y. Suezer, 35
G. Sutter, 35
E. Tuomanen, 11
J. Ulmer, 26
D. Urquiza, 32
J.-F. Viret, 20
J. Vollmer, 40
H. M. Vordermeier, 12, 31
H. Wedemeyer, 9
S. Weiss, 25
N. Westerfeld, 13
A. O. Whelan, 12
A. M. Wolf, 38
R. Zurbriggen, 41

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Conference Program

Thursday, April 10, 2003

4:30 – 9 p.m. **Welcome, Introductory Remarks and Keynote lectures**
Alexander von Gabain: *"Vaccine Development: from Empirical Medicine to Molecularly Designed Therapy"*
Antonio Coutinho*: "An outsiders view on the problems of vaccinology"*
Michel Gréco: *"Vaccines in the 21st century: an exciting and difficult world"*

Friday, April 11, 2003

9 – 12:10 a.m. **Session I: "Mechanism underlying immune response against infectious diseases and cancer: what can we learn for vaccine development"**
Staffan Normak: *"Bacterial Pathogenicity and Innate Immune Responses"*
Shizuo Akira: *"TLR family as receptors linking innate and acquired immunity"*
Elke Jäger: *"Antigen-specific Immunotherapy in Malignant Diseases"*
Rolf Kiessling: *"The Her-2/neu oncogene as a target for tumor vaccination"*
Rafi Ahmed: *"Immunological Memory: Remembering our pathogens"*

1:40 – 6 p.m. **Session II: "Novel vaccines for infectious diseases"**
Peter J. Openshaw: *"Problems and Prospects for RSV Vaccine Development"*
Michael P. Manns*: " Present and Future Concepts for Prophylactic and Therapeutic Vaccines against Hepatitis C "*
Noel Barrett: *"Development of a Novel Cell Culture-Derived Influenza Vaccine"*
Elaine Tuomanen: *"Pneumococcus: a shared paradigm for respiratory pathogens"*
Martin Vordermeier*: „Recognition of identical promiscuous determinates by T cells from different mammalian species and the use of bioinformatics to predict bovine T cell epitopes"*
Hermann Bujard: *"A candidate for a malaria vaccine: the merozoite surface protein 1 of Plasmodium falciparum"*
Jeffrey Almond: *"Vector approaches to vaccination against infectious diseases and cancer"*

Saturday, April 12, 2003

8 – 9:40 a.m. **Session III: "Cancer vaccines"**
Georg Stingl: *"Cell-based and molecular vaccines against melanoma – studies in man and experimental animals"*
Kathrin Jansen: *"Status of Prophylactic Vaccines against Cervical Cancer"*
Hans Loibner: "*Therapeutic vaccination against epithelial cancers with surrogateantigen vaccines: preclinical and clinical results*"

10:10 – 12:10 p.m. **Session IV: "Emerging topics in vaccines"**
Erik Depla: *"E1 vaccination in chronic HCV patients: Well-Tolerated and Possible Halting of Fibrosis Progression in Patients"*
Martin Bachmann: *"Virus-like particles: Combining Innate and Adaptive Immunity for Effective Vaccination"*
Jean-Francois Viret: *„Recombinant live oral vaccines against bacterial enteric pathogens: the way through regulation"*
Brian Barber: *"Delivering T-cell Immunity with Heat Shock Proteins"*
Gregory Glenn: *"Transcutaneous Immunization: Skin Immunization Using a Patch"*

1:10 – 5:30 p.m. **Session V: „Novel vaccine strategies for infectious diseases and cancer"**
Antonio Lanzavecchia: *"Maintenance of serological memory"*
Bernard Moss: *"Poxvirus Vectors With and Without DNA Priming"*
Siegfried Weiss: *"Bacteria mediated DNA transfer for gene therapy and vaccination"*
Jeffrey Ulmer: *"Delivery systems for vaccines and adjuvants"*
Rino Rappuoli: *"Reverse vaccinology, a genome-based approach to vaccine development"*
Andreas Meinke: *"Identification of the "antigenome" – a novel tool for the design and development of subunit vaccines against bacterial pathogens"*
Michael Buschle: *"Novel Potent Adjuvants for the Induction of Cellular and Humoral Immune Responses"*

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